

REMARKS

Claims 1-83 are pending in the instant application. Claims 4-7, 10, 14-16, 20, 22, 23, 28, 29, 35, 43-65 and 69-74 are withdrawn from consideration for being drawn to a non-elected invention. Claims 1-3, 8-9, 11-13, 17-19, 21, 24-27, 30-34, 36-42, 66-68, and 75-83 are currently under examination. Claims 1, 17, 30, 80, and 82 are amended. Support for the amendments to claims 1 and 17 can be found throughout the specification, for example in paragraphs 27 and 76-77. Amendments to claims 30, 80, and 82 are to correct informalities in the claims and do not alter the scope of the claims. No new matter is added by the amendments.

Claim Objections

The Office Action has objected to claims 30, 80, and 82 for including informalities. Applicant thanks the Examiner for the careful reading of the claims and the suggestions provided in the Office Action. Applicant has amended the claims as set forth above to correct the informalities per the suggestion of the Examiner. Withdrawal of the objections is respectfully requested.

Rejection under 35 U.S.C. §112, ¶1 for Lack of Written Description Withdrawn

Applicant thanks the Examiner for the withdrawal of the rejection of claims 1-3, 8, 11-13, 17-19, 21, 24-27, 30-33, 36-42, and 66-68 for lack of written description based on the arguments presented in the prior response.

Rejection under 35 U.S.C. §112, ¶1 for Lack of Enablement Maintained

The Office Action maintains the rejection of claims 1-3, 8-9, 11-13, 17-19, 21, 24-27, 30-34, 36-42, 66-68, and 75-76, and has further rejected new claims 77-83 for not being enabled.

Applicant respectfully disagrees.

Applicant has amended independent claims 1 and 17 as set forth above to recite that the methods comprise **decreasing** ezrin activity to promote cell proliferation or formation of new blood vessels by **decreasing ezrin activity before, during, or**

after the mammal is exposed to conditions conducive to damaging blood vessels. The remaining independent claim in the application, claim 30, already includes the limitation of decreasing ezrin activity. The Office Action of January 23, 2008 states:

From the results in the instant disclosure, one skilled in the art ***would expect that decreasing ezrin activity should reduce the severity of blood vessel damage when a mammal is exposed to conditions conducive to damaging the blood vessels.*** However, the art teaches that complex biological systems are unpredictable, especially when there are many different pathways involved in processes such as modulation of endothelial cell proliferation in formation of new blood vessels or reducing severity of blood vessel damage in mammals.

In the response to the Office Action, Applicant pointed to the animal data provided in Example 14 of the specification as an example of a complex biological system, i.e., a mouse. Applicant submitted, and sustains, that the in vitro data in combination with the in vivo data support the claimed invention.

The Office Action issued on October 1, 2008 states:

However, as stated in the previous office actions, the results of these studies and derived from in vitro experiments or experiments in which HUVECs were administered to a mammal, rather than direct administration of any specific inhibitor. While these results may lead one of skill in the art to conclude that inhibition of ezrin would promote endothelial cell proliferation or angiogenesis, one of ordinary skill in the art would also know that previous studies have indicated that administration of the Rho kinase inhibitor Y27632 produced opposite results.

Applicant submits that the Examiner requires an inappropriately high level of certainty and that the enablement rejection is inappropriate. The acknowledgement of the Examiner that the results provided might lead one to conclude that inhibition of ezrin would promote endothelial cell proliferation or angiogenesis is sufficient to meet the requirement for enablement.

Applicant points to the Training materials for Examining Patent Applications with Respect to 35 U.S.C. Section 112, First Paragraph- Enablement of Chemical/ Biotechnical Applications (available at

<http://www.uspto.gov/web/offices/pac/dapp/1pecba.htm#iib1>). Applicant specifically points to Section III(A)(2)(c)(ii) on the correlation of in vitro and in vivo data to the claims which is reproduced, in part, below:

Since the initial burden is on the examiner to give reasons for the lack of enablement, when possible to support by evidence, the examiner must also give reasons for a conclusion of lack of correlation for an in vitro or in vivo animal model example. A rigorous or an invariable exact correlation is not required, as stated in *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 USPQ 739, 747 (Fed. Cir. 1985):

based upon the relevant evidence as a whole, there is a reasonable correlation between the disclosed in vitro utility and an in vivo activity, and therefore a rigorous correlation is not necessary *where the disclosure of pharmacological activity is reasonable based upon the probative evidence.* (Citations omitted.) [emphasis added]

As noted by the Examiner, the claimed pharmacological activity is "reasonable based on the probative evidence" as required. Withdrawal of the rejection is respectfully requested.

Further, Applicant submits that the Office Action cites references that are either not sufficient to support the position of the Office Action or are not relevant to the claims under examination. The specification teaches the following:

We have discovered that it possible to modulate cells by changing activity of the ezrin protein. More specifically, we have found that under certain conditions ezrin associates with nucleic acid to impact cell proliferation. Without wishing to be bound to theory, it is believed that ezrin is a new nucleic acid binding protein that can modulate transcription of the cyclin A gene: a key cell cycle regulator. (page 4, lines 26-30).

Claim 1 is drawn to cell proliferation, which is a distinct and early step of new vessel formation as claimed in claim 17. Prompt reendothelialization decreases the severity of blood vessel damage under conditions conducive to damage as in claim 30. Cell proliferation and/or recruitment must occur prior to blood vessel formation. It was known at the time of filing of the instant application that TNF mediates cell cycle arrest. Cyclin A is a cell cycle regulatory gene/protein. Specifically, the specification states:

It has been disclosed that blocking TNF improves re-endothelialization after balloon angioplasty. Moreover, in vitro exposure of primary ECs to TNF has been reported to inhibit proliferation and enhance apoptosis. See Krasinski, K. et al. (2001), *supra*.

There have been efforts to understand how TNF impacts cell function. For example, it has been reported that TNF mediates EC cell cycle arrest. Cell cycle regulatory genes, including cyclin A, are thought to play a significant role. See Krasinski, K. et al. (2001), *supra*.

Cyclin A gene mRNA levels are thought to increase in S phase. The promoter of this gene is believed to harbor regulatory elements that facilitate cell cycle control and transcription of the gene. The transcriptional control elements are thought to include two repressor binding sites: a cell cycle dependent element (CDE) and a cell cycle gene homology region (CHR). See Beutler B. *J Investig Med.* 1995;43:227-35; Liu, N. et al. *Nucleic Acids Res.* 1997;25 :4915-20; and references cited therein. (page 3, lines 4-20)

The specification clearly demonstrates that inhibition of ezrin inhibits TNF inhibition of transcription through the Cyclin A promoter, preventing TNF from mediating cell cycle arrest. As noted above, balloon angioplasty and other conditions conducive to conducive to damaging blood vessels result in TNF inhibition of cell proliferation. Ezrin inhibitors can act to counter this inhibition by TNF.

The Office Action then proceeds to cite a number of references to demonstrate that the Rho kinase inhibitor Y-27632 inhibited angiogenesis, rather than promoted angiogenesis, as claimed herein. Applicant submits that the references cannot properly be used to create doubt around the conclusions arrived at by the inventors based on the data provided in the instant application.

First, the Office Action points to Figures 1-3 of Uchida. The Figure legends of Figures 1-3 read, in part:

FIG. 1. The effects of the CY3 or Y-27632 on the formation of capillary-like tubes were investigated. (A) After 24 h, the *HUVECs were cultured on Matrigel....*

FIG. 2. *Double fluorescent staining of the HUVECs* with rhodamine phalloidin and antivinculin antibody, with anti phosphotyrosine and anti-vinculin antibodies...

FIG. 3. (d) The dorsal skin of a mouse that was not fed Y-27632, on which the diffusion chamber including 1.0×10^6 cells of HT1080 cells had been placed. A round ring was replaced onto the tissues. Three groups of spiral vessels were seen developing transversely from preexisting vessels in the are [sic]. (e) The dorsal skin of a mouse, which was fed and administered Y-27632, on which the diffusion chamber including 1.0×10^6 cells of HT1080 cells had been placed. A round ring was replaced onto the tissues, too. Spiral vessels were not seen developing in the are [sic]. (f) The immunohistochemically stained vessels using anti-human factor related antigen-antibody in the submuscular layer of the mouse dorsal skin, not fed Y-27632, (g) fed and administered Y-27632 (magnification, x200) (the arrowheads are positively stained endothelial cells)....

Figures 1 and 2 are related to growing HUVEC cells in culture which cannot create doubt regarding the conclusions based on *in vivo* studies. In Figure 3, the methods conflict with the results section and make the analysis of the data unclear. The methods section teaches that the Y-27632 was administered interperitoneally at 100 mg/kg (first paragraph, left hand column, page 635). The description of the experiments in the results section states that Y-27632 was administered orally (first incomplete paragraph, right hand column, page 635). Were the animals used in the experiments that were "not fed" Y-27632 administered the composition by another method? As one skilled in the art could not understand the experiments performed, one would not draw a conclusion based on the results presented.

Moreover, the data collection methods are specious, if not at least unorthodox. The end of the first paragraph of the left hand paragraph of page 635 states:

For histological analyses, *two samples which seemed to show the most angiogenic activity in the fields were taken from each skin area*, embedded in paraffin wax, sliced into 4- μ m-thick vertical section, and then stained with H&E.

This admitted data selection calls into question any statement of any meaning or statistical significance of any results observed.

Further, although the methods of the Ushida reference teach administration of Y-27632 by some uncertain method, the method of inducing angiogenesis was by

implantation of HT1080 cells in a Millipore chamber in the back of the mouse. HT1080 cells are fibrosarcoma cells that likely secrete growth factors making it unclear what factors may promote angiogenesis in that model. This is clearly distinct from the instantly claimed methods in which angiogenesis is induced before, during, or after the mammal is exposed to conditions conducive to damaging blood vessels.

The Xue reference is concerned with angiogenesis related to metastatic cancer, not angiogenesis that is induced before, during, or after the mammal is exposed to conditions conducive to damaging blood vessels as presently claimed.

Hopkins et al., 2007 (Organized migration of epithelial cells requires control of adhesion and protrusion through Rho kinase effectors. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292:G806-817, copy enclosed) teaches that migration of non-endothelial cells is a distinct process from the migration of other cell types. Specifically, the reference states:

Migration of epithelial cells is fundamental to both physiological and pathophysiological processes. Since epithelia by nature form biological barriers in which individual cells must tightly associate with each other, ***epithelial cell migration is thus distinct from that of unattached/ single cells*** (e.g., leukocytes and fibroblasts) due to the propensity of epithelial cells to migrate in a cohesive unit. (page G812, first paragraph of Discussion)

Applicant submits that the teachings of Xue which are drawn to migration of non-endothelial cells is not relevant to the claimed methods that require endothelial cells.

The Office Action cites Hata et al to demonstrate that a Rho kinase inhibitor was found to inhibit angiogenesis in a mouse corneal pocket assay. ***Applicant submits that when performing the mouse corneal pocket assay, efforts are made to limit damage to the cornea, which is an avascular tissue, to prevent an inflammatory or other response and to allow for analysis of the activity of a single factor.*** The instant claims require that the method be performed in a mammal before, during, or after the mammal is exposed to conditions conducive to damaging blood vessels (i.e. in vascular tissue).

The Office Action also cites Van Nieuw Amerongen for teaching that cell migration is inhibited by Y-27362 in an *in vitro* wounding model. Applicant notes that the abstract states that:

Inhibition of Rho kinase prevented VEGF-enhanced EC-migration in response to mechanical wounding, ***but had no effect on basal EC migration.***

Therefore, Van Nieuw Amerongen teaches that ***enhanced*** cell migration is inhibited by Rho inhibition, not ***basal*** EC migration. The Office Action notes that angiogenesis cannot occur without migration. Applicant notes that angiogenesis cannot occur without cell proliferation.

Further, the Hopkins reference cited above notes the limitations of wound closing assays, stating:

[I]t must be remembered that cell motility in wound closure models is essentially a two dimensional process, whereas cell motility *in vivo* can occur in a two dimensional or three dimensional matrix where, in addition to matrix degradation, cells receive stimuli from the entire surface. (page G816, left hand column, first full paragraph)

Further, the Hopkins reference notes that administration of ROCK inhibitors has different effects on different cell types, albeit in *in vitro* assays:

[T]he effects of interfering with F-actin cytoskeletal organization through manipulation of ROCK may differ depending on the type and site of cells. Indeed, ROCK inhibition has been reported to increase the invasion and motility of astrocytoma cells through activation of Rac1 (41), but the balance of published evidence suggests that the inhibition of ROCK greatly reduces invasion in diverse cancerous epithelial types in Boyden chamber-based assays (2, 20, 26, 31, 42). (page G816, left hand column, first full paragraph)

Therefore, it is well understood in the art that depending on the specific condition and subject, a compound can have different effects. Applicant provides herewith a copy of Wolfrum et al., 2004 (Inhibition of Rho-kinase leads to rapid activation of phosphatidylinositol 3-kinase/protein kinase Akt cardiovascular protection. *Arterioscler. Thromb. Vasc. Biol.* 24:1842-1847) which demonstrates that inhibition of rho kinase, which in turn results in the inhibition of ezrin, provides cardiovascular

protection in two ischemia reperfusion injury models, one of which was myocardial ischemia which causes damage to blood vessels.

Applicant submits that as clearly demonstrated by the specification, upon vascular injury, which includes inflammation and TNF release, decreasing ezrin activity increases cell proliferation. This is clearly distinct from the cited art in which the activity of ezrin inhibitors were tested in the absence of inflammation.

Withdrawal of the rejections for lack of enablement is respectfully requested.

A request for an extension of two (2) months in time for reply is hereby requested. The Commissioner is hereby authorized to charge Deposit Account 04-1105 referencing Docket No: 58098(71417), the fee for the extension and a Request for Continued Examination, small entity. It is believed that no further fee is due. However, if a fee is due, the Commissioner is hereby authorized to charge the Deposit Account named above. Applicant requests that any overpayments be credited to the Account.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

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Respectfully submitted,

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